

A34

## (12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization  
International Bureau(43) International Publication Date  
9 January 2003 (09.01.2003)

PCT

(10) International Publication Number  
WO 03/002107 A2(51) International Patent Classification<sup>7</sup>: A61K 31/00,  
31/404, 31/505, 31/506, 31/519, 31/517

(21) International Application Number: PCT/IB02/03298

(22) International Filing Date: 28 June 2002 (28.06.2002)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:  
60/301,409 29 June 2001 (29.06.2001) US(71) Applicant (for all designated States except US): AB SCI-  
ENCE [FR/FR]; 3, avenue George V, F-75008 Paris (FR).

(72) Inventors; and

(75) Inventors/Applicants (for US only): MOUSSY, Alain  
[FR/FR]; 22 bis, Passage Dauphine, F-75006 Paris (FR).  
KINET, Jean-Pierre [FR/US]; 3 Hunt Road, Lexington,  
MA 02421 (US).(74) Agents: MARTIN, Jean-Jacques et al.; Cabinet Regim-  
beau, 20, rue de Chazelles, F-75847 Paris Cedex 17 (FR).(81) Designated States (national): AE, AG, AL, AM, AT, AU,  
AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU,  
CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH,  
GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC,  
LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW,  
MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG,  
SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ,  
VN, YU, ZA, ZM, ZW.(84) Designated States (regional): ARIPO patent (GH, GM,  
KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW),  
Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM),  
European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR,  
GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent  
(BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR,  
NE, SN, TD, TG).

## Declarations under Rule 4.17:

- as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii)) for the following designations AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZM, ZW, ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG)
- as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii)) for all designations
- of inventorship (Rule 4.17(iv)) for US only

## Published:

- without international search report and to be republished upon receipt of that report

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: USE OF TYROSINE KINASE INHIBITORS FOR TREATING MULTIPLE SCLEROSIS (MS)

(57) Abstract: The present invention relates to a method for treating Multiple Sclerosis (MS) comprising administering a tyrosine kinase inhibitor to a human in need of such treatment, more particularly a non-toxic, selective and potent c-kit inhibitor. Preferably said inhibitor is unable to promote death of IL-3 dependent cells cultured in presence of IL-3.

WO 03/002107 A2

WO 03/002107

PCT/IB02/03298

**Use of tyrosine kinase inhibitors for treating Multiple Sclerosis (MS)**

The present invention relates to a method for treating Multiple Sclerosis (MS) comprising administering a tyrosine kinase inhibitor to a human in need of such treatment, more particularly a non-toxic, selective and potent c-kit inhibitor. Preferably, said inhibitor is unable to promote death of IL-3 dependent cells cultured in presence of IL-3.

10 Multiple Sclerosis (MS) was first described in 1868 and is a highly variable disease, which usually begins between the second and fifth decades of life. MS is the most common disabling neurological disorder affecting young white adults. At least 350,000 Americans have MS, with women affected twice as often as men. MS usually starts by the ages of 15 and 50; the average age of onset is 30. Nearly all MS patients suffer such  
15 symptoms as fatigue, spasticity, tremor, decreased mobility, depression, pain, urologic complications, and cognitive impairment at some point during the course of their disease.

The most common signs of multiple sclerosis are sensory and visual motor dysfunction. MS involves lesions of 1 to 4 cm called plaques scattered throughout the white matter of  
20 the central nervous system. During the early evolution of the plaque, perivascular inflammatory cells (lymphocytes, plasma cells, macrophages) invade the substance of the white matter and are thought to play a critical role in myelin destruction. This process is followed by extensive gliosis by astrocytes and aberrant attempts at  
25 remyelination with oligodendrocytes proliferating at the edges of the plaque. In addition, immunoglobulins are deposited with each plaque. The chronic inflammatory autoimmune reactions responsible for this disease are reviewed in Steinman, et al.,

WO 03/002107

PCT/IB02/03298

2

Annu. Rev. Neurosci. 17:247, 1993; Miller, S. D. et al., Immunol. Today 15:356, 1994; French-Constant, C., Lancet 343:271-274, 1994; Brocke, S., et al., Trends in Microbiol. 2:250, 1994.

Furthermore, it is thought that the destruction of myelin may result from an immune  
5 attack directed against self or against novel antigen plus self, which is triggered by a  
virus, reviewed in Rodriguez, Multiple Sclerosis: basic concepts and hypothesis, Mayo  
Clin. Proc., 64:570-6 (1989). Viruses from many families and subfamilies  
(Herpetoviridae, Coronaviridae, Picornaviridae, Lentiviridae, Paramyxoviridae,  
Togaviridae) experimentally induce demyelination in animals of various species (e.g.,  
10 mice, rats, dogs, sheep). Dal Canto et al., Ann. Neurol., 11:109 (1982). These  
observations strengthen the idea that the immune system is involved in the mechanisms  
leading to the destruction of myelin.

In this regard, the infiltrating CD4 T-cells (Th1 cells) produce pro-inflammatory  
15 cytokines (interleukin (IL)-2, interferon (IFN)-gamma, and tumor necrosis factor  
(TNF)-alpha.) that activate antigen-presenting cells like macrophage to produce  
inflammatory cytokines (IL-1-beta, IL-6, and IL-8) and IL-12. The IL-12 induces further  
IFN-gamma synthesis. In this cyclical manner, a chronic autoantigen-driven immune  
reaction is thought to produce a demyelinating attack on the CNS.

20

Several therapeutic approaches have been proposed in the art to limit the immune-  
mediated CNS damage in MS by targeting the effector functions of activated Th1 cells  
and macrophages. Immunosuppressive drugs constitute the majority of agents currently  
used and under study and are reviewed in Noseworthy, J. H., "Immunosuppressive  
25 therapy in multiple sclerosis: pros and cons," International MS Journal 1:79-89, 1994.  
Examples are adrenocorticotrophic hormone, corticosteroid, prednisone,  
methylprednisone, 2-chlorodeoxyadenosine (Cladribine), mitoxantrone, sulphasalazine,

WO 03/002107

PCT/IB02/03298

3

methotrexate, total lymphoid irradiation, and interferon-beta. However, there are risks of infection during non-specific immunosuppression and the toxic side effects of some of the cytotoxic drugs are unsuitable with clinical treatments. Moreover, none of such treatments has been shown to be efficient as of today.

5

Consequently, the problem is to find alternative therapies that are better in terms of safety versus efficacy.

Mast cells (MC) are tissue elements derived from a particular subset of hematopoietic stem cells that express CD34, c-kit and CD13 antigens (Kirshenbaum et al, Blood. 94: 2333-2342, 1999 and Ishizaka et al, Curr Opin Immunol. 5: 937-43, 1993). Immature MC progenitors circulate in the bloodstream and differentiate in tissues. These differentiation and proliferation processes are under the influence of cytokines, one of utmost importance being Stem Cell Factor (SCF), also termed Kit ligand (KL), Steel factor (SL) or Mast Cell Growth Factor (MCGF). SCF receptor is encoded by the protooncogene c-kit, that belongs to type III receptor tyrosine kinase subfamily (Boissan and Arock, J Leukoc Biol. 67: 135-48, 2000). This receptor is also expressed on others hematopoietic or non hematopoietic cells. Ligation of c-kit receptor by SCF induces its dimerization followed by its transphosphorylation, leading to the recruitment and activation of various intracytoplasmic substrates. These activated substrates induce multiple intracellular signaling pathways responsible for cell proliferation and activation (Boissan and Arock, 2000). Mast cells are characterized by their heterogeneity, not only regarding tissue location and structure but also at the functional and histochemical levels (Aldenberg and Enerback., Histochem. J. 26: 587-96, 1994 ; Bradding et al. J Immunol. 155: 297-307, 1995 ; Irani et al, J Immunol. 147: 247-53, 1991 ; Miller et al, Curr Opin Immunol. 1: 637-42, 1989 and Welle et al, J Leukoc Biol. 61: 233-45, 1997).

WO 03/002107

PCT/IB02/03298

4

- Mast cells contain a variety of inflammatory mediators (serotonin, histamine and vasoactive peptides) and can release arachidonic acid metabolites of both the leukotrienes and the prostaglandin series. As a result, mast cells can be considered as gatekeepers of CNS inflammation. Indeed, these molecules have powerful effects on
- 5 local blood flow and vascular permeability. By this effect, mast cells could play an important role in promoting the entry of autoreactive T cells across the blood/brain barrier. In addition, Askenase et al. (1983), *J Exp Med* 157: 862-873 demonstrated that activated T cells are able to secrete an antigen-specific-factor that binds to mast cells and induce degranulation. Ibrahim et al. (1974), *J Neurol Sci.* 21: 431-478 demonstrated that
- 10 mast cells are present in the CNS of mammalian brain and are usually associated with blood vessels. This is further supported by the fact that vasoactive peptides (histamine and serotonin) have been demonstrated to be present in CNS mast cells (Edvinsson et al. 1977, *Neurology* 27: 878-883).
- 15 More recently, mast cells in and around demyelinating lesions in MS brain and not in control brain tissue were observed by Kruger et al. (1990): Mast cells and multiple sclerosis: A light and electron microscopic study of mast cells in multiple sclerosis emphasizing staining procedure. *Acta Neurol Scand* 81: 31-36.
- Ibrahim et al. (1996), *J of Neuroimmunol* 70: 131-138 have also observed tryptase
- 20 positive cells (mast cells) within and around MS plaques, especially the chronic active.
- Moreover, it has been postulated that release of vasoactive amines, kallikreins, arachidonic acid metabolites, and cytokines by CNS mast cells may further promote inflammatory cell traffic into the CNS (Bebo BF Jr, Yong T, Orr EL, Linthicum DS (1996): Hypothesis: a possible role for mast cells and their inflammatory mediators in
- 25 the pathogenesis of autoimmune encephalomyelitis. *J Neurosci Res* 45: 340-348).

WO 03/002107

PCT/IB02/03298

5

Besides, using a myelin oligodendrocyte glycoprotein (MOG)-induced model of acute EAE, it has been shown that mast cell-deficient W/W(v) mice exhibit significantly reduced disease incidence, delayed disease onset, and decreased mean clinical scores when compared with their wild-type congenic littermates. No differences were observed  
5 in MOG-specific T and B cell responses between the two groups, indicating that a global T or B cell defect is not present in W/W(v) animals. Reconstitution of the mast cell population in W/W(v) mice restores induction of early and severe disease to wild-type levels, Secor VH, (2000), J Exp Med 191(5): 813-821.

10 Actually, mast cell-dependent mediators are categorized here into three groups: preformed granule-associated mediators (histamine, proteoglycans, and neutral proteases), lipid-derived mediators (prostaglandins, thromboxanes and leucotrienes), and various cytokines (IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-8, TNF- $\alpha$ , GM-CSF, MIP-1 $\alpha$ , MIP-1 $\beta$  and IFN- $\gamma$ ). Here, the release of such mediators by CNS mast cells is found to be  
15 directly or indirectly responsible for the presence of plaques infiltrating Th1 cells, ultimately leading to the onset of MS. A

A new route for treating MS is provided, which consists of destroying mast cells playing a role in MS pathogenesis. It has been found that tyrosine kinase inhibitors and more particularly c-kit inhibitors are especially suited to reach this goal.

20

### Description

The present invention relates to a method for treating Multiple Sclerosis comprising administering a tyrosine kinase inhibitor to a human in need of such treatment.

25

Tyrosine kinase inhibitors are selected for example from bis monocyclic, bicyclic or heterocyclic aryl compounds (WO 92/20642), vinylene-azaindole derivatives (WO

WO 03/002107

PCT/IB02/03298

6

94/14808) and 1-cyclopropyl-4-pyridyl-quinolones (US 5,330,992), Styryl compounds (US 5,217,999), styryl-substituted pyridyl compounds (US 5,302,606), seleoindoles and selenides (WO 94/03427), tricyclic polyhydroxylic compounds (WO 92/21660) and benzylphosphonic acid compounds (WO 91/15495), pyrimidine derivatives (US 5,521,184 and WO 99/03854), indolinone derivatives and pyrrol-substituted indolinones (US 5,792,783, EP 934 931, US 5,834,504, US 5,883,116, US 5,883,113, US 5,886,020, WO 96/40116 and WO 00/38519), as well as bis monocyclic, bicyclic aryl and heteroaryl compounds (EP 584 222, US 5,656,643 and WO 92/20642), quinazoline derivatives (EP 602 851, EP 520 722, US 3,772,295 and US 4,343,940) and aryl and heteroaryl quinazoline (US 5,721,237, US 5,714,493, US 5,710,158 and WO 95/15758).

Preferably, said tyrosine kinase inhibitors are unable to promote death of IL-3 dependent cells cultured in presence of IL-3.

15 In another embodiment, the invention is directed to a method for treating Multiple Sclerosis comprising administering a c-kit inhibitor to a human in need of such treatment.

Preferably, said c-kit inhibitor is a non-toxic, selective and potent c-kit inhibitor. Such inhibitors can be selected from the group consisting of indolinones, pyrimidine derivatives, pyrrolopyrimidine derivatives, quinazoline derivatives, quinoxaline derivatives, pyrazoles derivatives, bis monocyclic, bicyclic or heterocyclic aryl compounds, vinylene-azaindole derivatives and pyridyl-quinolones derivatives, styryl compounds, styryl-substituted pyridyl compounds, seleoindoles, selenides, tricyclic polyhydroxylic compounds and benzylphosphonic acid compounds.

25

Among preferred compounds, it is of interest to focus on pyrimidine derivatives such as N-phenyl-2-pyrimidine-amine derivatives (US 5,521,184 and WO 99/03854), indolinone derivatives and pyrrol-substituted indolinones (US 5,792,783, EP 934 931, US

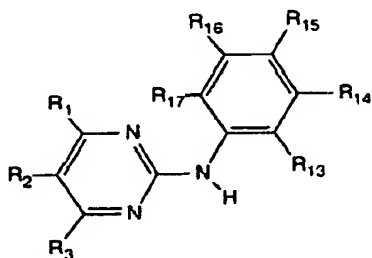
WO 03/002107

PCT/IB02/03298

7

5,834,504), US 5,883,116, US 5,883,113, US 5, 886,020, WO 96/40116 and WO 00/38519), as well as bis monocyclic, bicyclic aryl and heteroaryl compounds (EP 584 222, US 5,656,643 and WO 92/20642), quinazoline derivatives (EP 602 851, EP 520 722, US 3,772,295 and US 4,343,940), 4-amino-substituted quinazolines (US 3,470,182), 4-thienyl-2-(1H)-quinazolones, 6,7-dialkoxyquinazolines (US 3,800,039), aryl and heteroaryl quinazoline (US 5,721,237, US 5,714,493, US 5,710,158 and WO 95/15758), 4-anilinoquinazoline compounds (US 4,464,375), and 4-thienyl-2-(1H)-quinazolones (US 3,551,427).

- 10 So, preferably, the invention relates to a method for treating Multiple Sclerosis comprising administering a non toxic, potent and selective c-kit inhibitor. Such inhibitor can be selected from pyrimidine derivatives, more particularly N-phenyl-2-pyrimidine-amine derivatives of formula I :



- 15 wherein the R<sub>1</sub>, R<sub>2</sub>, R<sub>3</sub>, R<sub>13</sub> to R<sub>17</sub> groups have the meanings depicted in EP 564 409 B1, incorporated herein in the description.

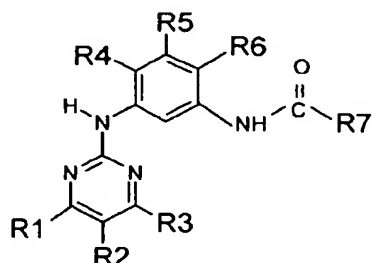
Preferably, the N-phenyl-2-pyrimidine-amine derivative is selected from the compounds corresponding to formula II :

20

WO 03/002107

PCT/IB02/03298

8



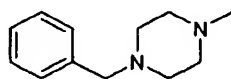
Wherein R1, R2 and R3 are independently chosen from H, F, Cl, Br, I, a C1-C5 alkyl or a cyclic or heterocyclic group, especially a pyridyl group;

- 5 R4, R5 and R6 are independently chosen from H, F, Cl, Br, I, a C1-C5 alkyl, especially a methyl group;

and R7 is a phenyl group bearing at least one substituent, which in turn possesses at least one basic site, such as an amino function.

Preferably, R7 is the following group :

10



Among these compounds, the preferred are defined as follows :

R1 is a heterocyclic group, especially a pyridyl group,

R2 and R3 are H,

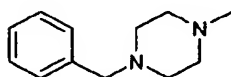
- 15 R4 is a C1-C3 alkyl, especially a methyl group,

R5 and R6 are H,

and R7 is a phenyl group bearing at least one substituent, which in turn possesses at least one

basic site, such as an amino function, for example the group :

20



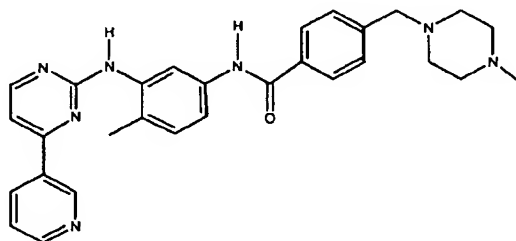
WO 03/002107

PCT/IB02/03298

9

Therefore, in a preferred embodiment, the invention relates to a method for treating Multiple Sclerosis comprising the administration of an effective amount of the compound known in the art as CGP57148B :

4-(4-méthylpipérazine-1-ylméthyl)-N-[4-méthyl-3-(4-pyridine-3-yl)pyrimidine-2  
5 ylamino]phényl]-benzamide corresponding to the following formula :



The preparation of this compound is described in example 21 of EP 564 409 and the  $\beta$ -form, which is particularly useful is described in WO 99/03854.

10

Alternatively, the c-kit inhibitor can be selected from :

- indolinone derivatives, more particularly pyrrol-substituted indolinones,
- monocyclic, bicyclic aryl and heteroaryl compounds, quinazoline derivatives,
- and quinaxolines, such as 2-phényl-quinaxoline derivatives, for example 2-phényl-  
15 6,7-dimethoxy quinaxoline.

In a preferred aspect, the invention contemplated the method mentioned above, wherein said c-kit inhibitor is unable to promote death of IL-3 dependent cells cultured in presence of IL-3.

20

In a further embodiment, c-kit inhibitors as mentioned above are inhibitors of activated c-kit. In frame with the invention, the expression "activated c-kit" means a constitutively activated-mutant c-kit including at least one mutation selected from point mutations,

WO 03/002107

PCT/IB02/03298

10

- deletions, insertions, but also modifications and alterations of the natural c-kit sequence (SEQ ID N°1). Such mutations, deletions, insertions, modifications and alterations can occur in the transphosphorylase domain, in the juxtamembrane domain as well as in any domain directly or indirectly responsible for c-kit activity. The expression “activated c-kit” also means herein SCF-activated c-kit. Preferred and optimal SCF concentrations for activating c-kit are comprised between  $5.10^{-7}$  M and  $5.10^{-6}$  M, preferably around  $2.10^{-6}$  M. In a preferred embodiment, the activated-mutant c-kit in step a) has at least one mutation proximal to Y823, more particularly between amino acids 800 to 850 of SEQ ID No1 involved in c-kit autophosphorylation, notably the D816V, D816Y, D816F and D820G mutants. In another preferred embodiment, the activated-mutant c-kit in step a) has a deletion in the juxtamembrane domain of c-kit. Such a deletion is for example between codon 573 and 579 called c-kit d(573-579). The point mutation V559G proximal to the juxtamembrane domain c-kit is also of interest.
- 15 In this regard, the invention contemplates a method for treating Multiple Sclerosis comprising administering to a human in need of such treatment a compound that is a selective, potent and non toxic inhibitor of activated c-kit obtainable by a screening method which comprises :
- a) bringing into contact (i) activated c-kit and (ii) at least one compound to be tested;
  - 20 under conditions allowing the components (i) and (ii) to form a complex,
  - b) selecting compounds that inhibit activated c-kit,
  - c) testing and selecting a subset of compounds identified in step b), which are unable to promote death of IL-3 dependent cells cultured in presence of IL-3.
- 25 This screening method can further comprise the step consisting of testing and selecting a subset of compounds identified in step b) that are inhibitors of mutant activated c-kit (for example in the transphosphorylase domain), which are also capable of inhibiting SCF-activated c-kit wild.

WO 03/002107

PCT/IB02/03298

11

Alternatively, in step a) activated c-kit is SCF-activated c-kit wild.

A best mode for practicing this method consists of testing putative inhibitors at a concentration above 10  $\mu$ M in step a). Relevant concentrations are for example 10, 15,  
5 20, 25, 30, 35 or 40  $\mu$ M.

In step c), IL-3 is preferably present in the culture media of IL-3 dependent cells at a concentration comprised between 0.5 and 10 ng/ml, preferably between 1 to 5 ng/ml.

10 Examples of IL-3 dependent cells include but are not limited to :

- cell lines naturally expressing and depending on c-kit for growth and survival. Among such cells, human mast cell lines can be established using the following procedures : normal human mast cells can be infected by retroviral vectors containing sequences coding for a mutant c-kit comprising the c-kit signal peptide and a TAG sequence  
15 allowing to differentiate mutant c-kits from c-kit wild expressed in hematopoietic cells by means of antibodies.

This technique is advantageous because it does not induce cellular mortality and the genetic transfer is stable and gives satisfactory yields (around 20 %). Pure normal human mast cells can be routinely obtained by culturing precursor cells originating from blood  
20 obtained from human umbilical vein. In this regard, heparinated blood from umbilical vein is centrifuged on a Ficoll gradient so as to isolate mononucleated cells from other blood components. CD34<sup>+</sup> precursor cells are then purified from the isolated cells mentioned above using the immunomagnetic selection system MACS (Miltenyi biotech). CD34<sup>+</sup> cells are then cultured at 37°C in 5 % CO<sub>2</sub> atmosphere at a concentration of 10<sup>5</sup>  
25 cells per ml in the medium MCCM ( $\alpha$ -MEM supplemented with L-glutamine, penicillin, streptomycin, 5 10<sup>-5</sup> M  $\beta$ -mercaptoethanol, 20 % veal foetal serum, 1 % bovine albumin

WO 03/002107

PCT/IB02/03298

12

serum and 100 ng/ml recombinant human SCF. The medium is changed every 5 to 7 days. The percentage of mast cells present in the culture is assessed each week, using May-Grünwal Giemsa or Toluidine blue coloration. Anti-tryptase antibodies can also be used to detect mast cells in culture. After 10 weeks of culture, a pure cellular population  
5 of mast cells (< 98 %) is obtained.

It is possible using standard procedures to prepare vectors expressing c-kit for transfecting the cell lines established as mentioned above. The cDNA of human c-kit has been described in Yarden et al., (1987) EMBO J.6 (11), 3341-3351. The coding part of c-kit (3000 bp) can be amplified by PCR and cloned, using the following  
10 oligonucleotides :

- 5'AAGAAGAGATGGTACCTCGAGGGGTGACCC3' (SEQ ID No2) sens
- 5'CTGCTTCGCGGCCGCGTTAACTCTTCTCAACCA3' (SEQ ID No3) antisens

The PCR products, digested with NotI and XhoI, has been inserted using T4 ligase in  
15 the pFlag-CMV vector (SIGMA), which vector is digested with NotI and XhoI and dephosphorylated using CIP (Biolabs). The pFlag-CMV-c-kit is used to transform bacterial clone XLI-blue. The transformation of clones is verified using the following primers :

- 5'AGCTCGTTTAGTGAACCGTC3' (SEQ ID No4) sens,
- 20 - 5'GTCAGACAAAATGATGCAAC3' (SEQ ID No5) antisens.

Directed mutagenesis is performed using relevant cassettes is performed with routine and common procedure known in the art..

The vector Migr-I (ABC) can be used as a basis for constructing retroviral vectors used for transfecting mature mast cells. This vector is advantageous because it contains the  
25 sequence coding for GFP at the 3' and of an IRES. These features allow to select cells

WO 03/002107

PCT/IB02/03298

13

infected by the retrovirus using direct analysis with a fluorocytometer. As mentioned above, the N-terminal sequence of c-kit c-DNA can be modified so as to introduce a Flag sequence that will be useful to discriminating heterogeneous from endogenous c-kit.

5 Other IL-3 dependent cell lines that can be used include but are not limited to:

- BaF3 mouse cells expressing wild-type or mutated form of c-kit (in the juxtamembrane and in the catalytic sites) are described in Kitayama et al, (1996), Blood 88, 995-1004 and Tsujimura et al, (1999), Blood 93, 1319-1329.
- IC-2 mouse cells expressing either c-kit<sup>WT</sup> or c-kit<sup>D814Y</sup> are presented in Piao et al,  
10 (1996), Proc. Natl. Acad. Sci. USA 93, 14665-14669.

IL-3 independent cell lines are :

- HMC-1, a factor-independent cell line derived from a patient with mast cell leukemia, expresses a juxtamembrane mutant c-kit polypeptide that has constitutive kinase activity  
15 (Furitsu T et al, J Clin Invest. 1993;92:1736-1744 ; Butterfield et al, Establishment of an immature mast cell line from a patient with mast cell leukemia. Leuk Res. 1988;12:345-355 and Nagata et al, Proc Natl Acad Sci U S A. 1995;92:10560-10564).
- P815 cell line (mastocytoma naturally expressing c-kit mutation at the 814 position) has been described in Tsujimura et al, (1994), Blood 83, 2619-2626.

20

The extent to which component (ii) inhibits activated c-kit can be measured *in vitro* or *in vivo*. In case it is measured *in vivo*, cell lines expressing an activated-mutant c-kit, which has at least one mutation proximal to Y823, more particularly between amino acids 800 to 850 of SEQ ID No1 involved in c-kit autophosphorylation, notably the D816V,  
25 D816Y, D816F and D820G mutants, are preferred.

Example of cell lines expressing an activated-mutant c-kit are as mentioned.

WO 03/002107

PCT/IB02/03298

14

In another preferred embodiment, the method further comprises the step consisting of testing and selecting compounds capable of inhibiting c-kit wild at concentration below 1  $\mu$ M. This can be measured *in vitro* or *in vivo*.

5

Therefore, compounds are identified and selected according to the method described above are potent, selective and non-toxic c-kit wild inhibitors.

Alternatively, the screening method as defined above can be practiced *in vitro*. In this regard, the inhibition of mutant-activated c-kit and/or c-kit wild can be measured using standard biochemical techniques such as immunoprecipitation and western blot. Preferably, the amount of c-kit phosphorylation is measured.

In a still further embodiment, the invention contemplates a method for treating Multiple Sclerosis as depicted above wherein the screening comprises :

- a) performing a proliferation assay with cells expressing a mutant c-kit (for example in the transphosphorylase domain), which mutant is a permanent activated c-kit, with a plurality of test compounds to identify a subset of candidate compounds targeting activated c-kit, each having an  $IC_{50} < 10 \mu$ M, by measuring the extent of cell death,
- b) performing a proliferation assay with cells expressing c-kit wild said subset of candidate compounds identified in step (a), said cells being IL-3 dependent cells cultured in presence of IL-3, to identify a subset of candidate compounds targeting specifically c-kit,
- c) performing a proliferation assay with cells expressing c-kit, with the subset of compounds identified in step b) and selecting a subset of candidate compounds targeting c-kit wild, each having an  $IC_{50} < 10 \mu$ M, preferably an  $IC_{50} < 1 \mu$ M, by measuring the extent of cell death.

WO 03/002107

PCT/IB02/03298

15

Here, the extent of cell death can be measured by <sup>3</sup>H thymidine incorporation, the trypan blue exclusion method or flow cytometry with propidium iodide. These are common techniques routinely practiced in the art.

- 5 The method according to the invention includes preventing, delaying the onset and/or treating Multiple Sclerosis.

Therefore, the invention embraces the use of the compounds defined above to manufacture a medicament for treating Multiple Sclerosis.

10

The pharmaceutical compositions utilized in this invention may be administered by any number of routes including, but not limited to, oral, intravenous, intramuscular, intra-arterial, intramedullary, intrathecal, intraventricular, transdermal, subcutaneous, intraperitoneal, intranasal, enteral, topical, sublingual, or rectal means.

15

- In addition to the active ingredients, these pharmaceutical compositions may contain suitable pharmaceutically-acceptable carriers comprising excipients and auxiliaries which facilitate processing of the active compounds into preparations which can be used pharmaceutically. Further details on techniques for formulation and administration may  
20 be found in the latest edition of Remington's Pharmaceutical Sciences (Maack Publishing Co., Easton, Pa.).

- Pharmaceutical compositions for oral administration can be formulated using pharmaceutically acceptable carriers well known in the art in dosages suitable for oral  
25 administration. Such carriers enable the pharmaceutical compositions to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions, and the like, for ingestion by the patient.

WO 03/002107

PCT/IB02/03298

16

Pharmaceutical preparations for oral use can be obtained through combination of active compounds with solid excipient. Suitable excipients are carbohydrate or protein fillers, such as sugars, including lactose, sucrose, mannitol, or sorbitol; starch from corn, wheat, rice, potato, or other plants; cellulose, such as methyl cellulose, hydroxypropylmethyl-  
5 cellulose, or sodium carboxymethylcellulose; gums including arabic and tragacanth; and proteins such as gelatin and collagen. If desired, disintegrating or solubilizing agents may be added, such as the cross-linked polyvinyl pyrrolidone, agar, alginic acid, or a salt thereof, such as sodium alginate.

10 Dragee cores may be used in conjunction with suitable coatings, such as concentrated sugar solutions, which may also contain gum arabic, talc, polyvinylpyrrolidone, carbopol gel, polyethylene glycol, and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures. Dyestuffs or pigments may be added to the tablets or dragee coatings for product identification or to characterize the quantity of active  
15 compound, i.e., dosage.

Pharmaceutical preparations which can be used orally include capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a coating, such as glycerol or sorbitol. Push-fit capsules can contain active ingredients mixed with a filler or binders,  
20 such as lactose or starches, lubricants, such as talc or magnesium stearate, and, optionally, stabilizers. In soft capsules, the active compounds may be dissolved or suspended in suitable liquids, such as fatty oils, liquid, or liquid polyethylene glycol with or without stabilizers.

25 Pharmaceutical formulations suitable for parenteral administration may be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hanks' solution, Ringer's solution, or physiologically buffered saline. Aqueous injection suspensions may contain substances which increase the viscosity of the suspension, such

WO 03/002107

PCT/IB02/03298

17

as sodium carboxymethyl cellulose, sorbitol, or dextran. Additionally, suspensions of the active compounds may be prepared as appropriate oily injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils such as sesame oil, or synthetic fatty acid esters, such as ethyl oleate or triglycerides, or liposomes. Non-lipid polycationic amino  
5 polymers may also be used for delivery. Optionally, the suspension may also contain suitable stabilizers or agents which increase the solubility of the compounds to allow for the preparation of highly concentrated solutions.

The pharmaceutical composition may be provided as a salt and can be formed with many  
10 acids, including but not limited to, hydrochloric, sulfuric, acetic, lactic, tartaric, malic, and succine, acids, etc. Salts tend to be more soluble in aqueous or other protonic solvents than are the corresponding free base forms. In other cases, the preferred preparation may be a lyophilized powder which may contain any or all of the following: 1-50 mM histidine, 0. 1%-2% sucrose, and 2-7% mannitol, at a pH range of 4.5 to 5.5,  
15 that is combined with buffer prior to use.

Pharmaceutical compositions suitable for use in the invention include compositions wherein c-kit inhibitors are contained in an effective amount to achieve the intended purpose. The determination of an effective dose is well within the capability of those  
20 skilled in the art. A therapeutically effective dose refers to that amount of active ingredient, which ameliorates the symptoms or condition. Therapeutic efficacy and toxicity may be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., ED50 (the dose therapeutically effective in 50% of the population) and LD50 (the dose lethal to 50% of the population). The dose ratio of toxic  
25 to therapeutic effects is the therapeutic index, and it can be expressed as the ratio, LD50/ED50. Pharmaceutical compositions which exhibit large therapeutic indices are preferred. As mentioned above, a tyrosine kinase inhibitor and more particularly a c-kit

WO 03/002107

PCT/IB02/03298

18

inhibitor according to the invention is unable to promote death of IL-3 dependent cells cultured in presence of IL-3.

5

WO 03/002107

PCT/IB02/03298

19

**CLAIMS**

- 5 1. A method for treating Multiple Sclerosis comprising administering a tyrosine kinase inhibitor to a human in need of such treatment.
2. A method according to claim 1, wherein said tyrosine kinase inhibitor is unable to promote death of IL-3 dependent cells cultured in presence of IL-3.
- 10 3. A method for treating Multiple Sclerosis comprising administering a c-kit inhibitor to a human in need of such treatment.
4. A method according to claim 3, wherein said c-kit inhibitor is a non-toxic, selective  
15 and potent c-kit inhibitor.
5. A method according to claim 4, wherein said inhibitor is selected from the group consisting of indolinones, pyrimidine derivatives, pyrrolopyrimidine derivatives, quinazoline derivatives, quinoxaline derivatives, pyrazoles derivatives, bis monocyclic,  
20 bicyclic or heterocyclic aryl compounds, vinylene-azaindole derivatives and pyridyl-quinolones derivatives, styryl compounds, styryl-substituted pyridyl compounds, seleoindoles, selenides, tricyclic polyhydroxylic compounds and benzyolphosphonic acid compounds.
- 25 6. A method according to claim 4, wherein said inhibitor is selected from the group consisting of :
- pyrimidine derivatives, more particularly N-phenyl-2-pyrimidine-amine derivatives.
  - indolinone derivatives, more particularly pyrrol-substituted indolinones,

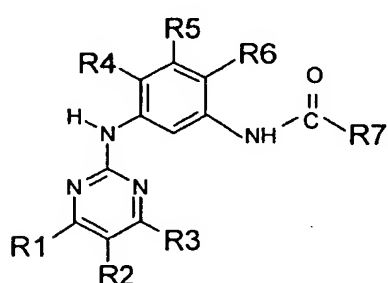
WO 03/002107

PCT/IB02/03298

20

- monocyclic, bicyclic aryl and heteroaryl compounds,
- and quinazoline derivatives.

7. A method according to one of claims 3 to 6, wherein said c-kit inhibitor is selected  
 5 from compounds of formula II :



Wherein R1, R2 and R3 are independently chosen from H, F, Cl, Br, I, a C1-C5 alkyl or  
 10 a cyclic or heterocyclic group, especially a pyridyl group;

R4, R5 and R6 are independently chosen from H, F, Cl, Br, I, a C1-C5 alkyl, especially a  
 methyl group;

and R7 is a phenyl group bearing at least one substituent, which in turn possesses at least  
 one basic site, such as an amino function.

15

8. A method according to one of claims 3 to 6, wherein said c-kit inhibitor is the  
 4-(4-méthylpipérazine-1-ylméthyl)-N-[4-méthyl-3-(4-pyridine-3-yl)pyrimidine-2  
 ylamino]phényl]-benzamide.

20

WO 03/002107

PCT/IB02/03298

21

9. A method according to one of claims 3 to 8, wherein said c-kit inhibitor is unable to promote death of IL-3 dependent cells cultured in presence of IL-3.

10. A method according to one of claims 3 to 9, wherein said c-kit inhibitor is an  
5 inhibitor of activated c-kit.

11. A method according to claim 10, wherein said activated c-kit inhibitor is capable of inhibiting SCF-activated c-kit.

10 12. A method according to claim 10, wherein said inhibitor is capable of inhibiting constitutively activated-mutant c-kit.

13. A method for treating Multiple Sclerosis comprising administering to a human in need of such treatment a compound that is a selective, potent and non toxic inhibitor of  
15 activated c-kit obtainable by a screening method which comprises :

a) bringing into contact (i) activated c-kit and (ii) at least one compound to be tested;  
under conditions allowing the components (i) and (ii) to form a complex,  
b) selecting compounds that inhibit activated c-kit,  
c) testing and selecting a subset of compounds identified in step b), which are unable to  
20 promote death of IL-3 dependent cells cultured in presence of IL-3.

14. A method according to claim 13, wherein the screening method further comprises the step consisting of testing and selecting a subset of compounds identified in step b) that are inhibitors of mutant activated c-kit, which are also capable of inhibiting SCF-  
25 activated c-kit wild.

15. A method according to claim 13, wherein activated c-kit is SCF-activated c-kit wild in step a).

WO 03/002107

PCT/IB02/03298

22

16. A method according to one of claims 13 to 15, wherein putative inhibitors are tested at a concentration above 10  $\mu$ M in step a).

5 17. A method according to one of claims 13 to 16, wherein IL-3 is preferably present in the culture media of IL-3 dependent cells at a concentration comprised between 0.5 and 10 ng/ml, preferably between 1 to 5 ng/ml.

18. A method according to claim 17, wherein IL-3 dependent cells are selected from the  
10 group consisting of mast cells, transfected mast cells, BaF3, and IC-2.

19. A method according to one of claims 13 to 18, wherein the extent to which component (ii) inhibits activated c-kit is measured *in vitro* or *in vivo*.

15 20. A method according to one of claims 13 to 19, further comprising the step consisting of testing and selecting compounds capable of inhibiting c-kit wild at concentration below 1  $\mu$ M.

21. A method according to claim 20, wherein the testing is performed *in vitro* or *in vivo*.

20

22. A method according to one of claims 13 to 21, wherein the inhibition of mutant-activated c-kit and/or c-kit wild is measured using standard biochemical techniques such as immunoprecipitation and western blot.

25 23. A method according to one of claims 13 to 21, wherein the amount of c-kit phosphorylation is measured.

WO 03/002107

PCT/IB02/03298

23

24. A method according to one of claims 13 to 23, wherein identified and selected compounds are potent, selective and non-toxic c-kit wild inhibitors.

25. A method for treating Multiple Sclerosis comprising administering to a human in  
5 need of such treatment a c-kit inhibitor obtainable by a screening method comprising :  
a) performing a proliferation assay with cells expressing a mutant c-kit (for example in  
the transphosphorylase domain), which mutant is a permanent activated c-kit, with a  
plurality of test compounds to identify a subset of candidate compounds targeting  
activated c-kit, each having an  $IC_{50} < 10 \mu M$ , by measuring the extent of cell death,  
10 b) performing a proliferation assay with cells expressing c-kit wild said subset of  
candidate compounds identified in step (a), said cells being IL-3 dependent cells cultured  
in presence of IL-3, to identify a subset of candidate compounds targeting specifically c-  
kit,  
c) performing a proliferation assay with cells expressing c-kit, with the subset of  
15 compounds identified in step b) and selecting a subset of candidate compounds targeting  
c-kit wild, each having an  $IC_{50} < 10 \mu M$ , preferably an  $IC_{50} < 1 \mu M$ , by measuring the  
extent of cell death.

26. A method according to claim 25, wherein the extent of cell death is measured by  $^3H$   
20 thymidine incorporation, the trypan blue exclusion method or flow cytometry with  
propidium iodide.

27. A method according to one of claims 1 to 26 for preventing, delaying the onset and  
treating Multiple Sclerosis.

25

28. Use of a c-kit inhibitor to manufacture a medicament for treating Multiple Sclerosis.

WO 03/002107

PCT/IB02/03298

1/5

## SEQUENCE LISTING

&lt;110&gt; AB Science

&lt;120&gt; Use of tyrosine kinase inhibitors for treating Multiple Sclerosis

&lt;130&gt; D19696 NT

&lt;150&gt; US 60/301,409

&lt;151&gt; 2001-06-29

&lt;160&gt; 5

&lt;170&gt; PatentIn Ver. 2.1

&lt;210&gt; 1

&lt;211&gt; 976

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;223&gt; Human c-kit

&lt;400&gt; 1

Met Arg Gly Ala Arg Gly Ala Trp Asp Phe Leu Cys Val Leu Leu Leu  
 1 5 10 15

Leu Leu Arg Val Gln Thr Gly Ser Ser Gln Pro Ser Val Ser Pro Gly  
 20 25 30

Glu Pro Ser Pro Pro Ser Ile His Pro Gly Lys Ser Asp Leu Ile Val  
 35 40 45

Arg Val Gly Asp Glu Ile Arg Leu Leu Cys Thr Asp Pro Gly Phe Val  
 50 55 60

Lys Trp Thr Phe Glu Ile Leu Asp Glu Thr Asn Glu Asn Lys Gln Asn  
 65 70 75 80

Glu Trp Ile Thr Glu Lys Ala Glu Ala Thr Asn Thr Gly Lys Tyr Thr  
 85 90 95

Cys Thr Asn Lys His Gly Leu Ser Asn Ser Ile Tyr Val Phe Val Arg  
 100 105 110

Asp Pro Ala Lys Leu Phe Leu Val Asp Arg Ser Leu Tyr Gly Lys Glu  
 115 120 125

Asp Asn Asp Thr Leu Val Arg Cys Pro Leu Thr Asp Pro Glu Val Thr  
 130 135 140

Asn Tyr Ser Leu Lys Gly Cys Gln Gly Lys Pro Leu Pro Lys Asp Leu  
 145 150 155 160

Arg Phe Ile Pro Asp Pro Lys Ala Gly Ile Met Ile Lys Ser Val Lys  
 165 170 175

Arg Ala Tyr His Arg Leu Cys Leu His Cys Ser Val Asp Gln Glu Gly  
 180 185 190

Lys Ser Val Leu Ser Glu Lys Phe Ile Leu Lys Val Arg Pro Ala Phe

WO 03/002107

PCT/IB02/03298

2/5

195					200					205					
Lys	Ala	Val	Pro	Val	Val	Ser	Val	Ser	Lys	Ala	Ser	Tyr	Leu	Leu	Arg
210						215					220				
Glu	Gly	Glu	Glu	Phe	Thr	Val	Thr	Cys	Thr	Ile	Lys	Asp	Val	Ser	Ser
225					230					235					240
Ser	Val	Tyr	Ser	Thr	Trp	Lys	Arg	Glu	Asn	Ser	Gln	Thr	Lys	Leu	Gln
				245					250					255	
Glu	Lys	Tyr	Asn	Ser	Trp	His	His	Gly	Asp	Phe	Asn	Tyr	Glu	Arg	Gln
			260					265					270		
Ala	Thr	Leu	Thr	Ile	Ser	Ser	Ala	Arg	Val	Asn	Asp	Ser	Gly	Val	Phe
		275					280					285			
Met	Cys	Tyr	Ala	Asn	Asn	Thr	Phe	Gly	Ser	Ala	Asn	Val	Thr	Thr	Thr
	290					295					300				
Leu	Glu	Val	Val	Asp	Lys	Gly	Phe	Ile	Asn	Ile	Phe	Pro	Met	Ile	Asn
305					310					315					320
Thr	Thr	Val	Phe	Val	Asn	Asp	Gly	Glu	Asn	Val	Asp	Leu	Ile	Val	Glu
				325					330					335	
Tyr	Glu	Ala	Phe	Pro	Lys	Pro	Glu	His	Gln	Gln	Trp	Ile	Tyr	Met	Asn
			340					345					350		
Arg	Thr	Phe	Thr	Asp	Lys	Trp	Glu	Asp	Tyr	Pro	Lys	Ser	Glu	Asn	Glu
		355					360					365			
Ser	Asn	Ile	Arg	Tyr	Val	Ser	Glu	Leu	His	Leu	Thr	Arg	Leu	Lys	Gly
		370				375					380				
Thr	Glu	Gly	Gly	Thr	Tyr	Thr	Phe	Leu	Val	Ser	Asn	Ser	Asp	Val	Asn
385					390					395					400
Ala	Ala	Ile	Ala	Phe	Asn	Val	Tyr	Val	Asn	Thr	Lys	Pro	Glu	Ile	Leu
				405					410					415	
Thr	Tyr	Asp	Arg	Leu	Val	Asn	Gly	Met	Leu	Gln	Cys	Val	Ala	Ala	Gly
			420					425					430		
Phe	Pro	Glu	Pro	Thr	Ile	Asp	Trp	Tyr	Phe	Cys	Pro	Gly	Thr	Glu	Gln
		435					440					445			
Arg	Cys	Ser	Ala	Ser	Val	Leu	Pro	Val	Asp	Val	Gln	Thr	Leu	Asn	Ser
		450				455					460				
Ser	Gly	Pro	Pro	Phe	Gly	Lys	Leu	Val	Val	Gln	Ser	Ser	Ile	Asp	Ser
465					470					475					480
Ser	Ala	Phe	Lys	His	Asn	Gly	Thr	Val	Glu	Cys	Lys	Ala	Tyr	Asn	Asp
				485					490					495	
Val	Gly	Lys	Thr	Ser	Ala	Tyr	Phe	Asn	Phe	Ala	Phe	Lys	Gly	Asn	Asn
			500					505					510		
Lys	Glu	Gln	Ile	His	Pro	His	Thr	Leu	Phe	Thr	Pro	Leu	Leu	Ile	Gly
		515					520					525			

WO 03/002107

PCT/IB02/03298

3/5

Phe Val Ile Val Ala Gly Met Met Cys Ile Ile Val Met Ile Leu Thr  
 530 535 540  
 Tyr Lys Tyr Leu Gln Lys Pro Met Tyr Glu Val Gln Trp Lys Val Val  
 545 550 555 560  
 Glu Glu Ile Asn Gly Asn Asn Tyr Val Tyr Ile Asp Pro Thr Gln Leu  
 565 570 575  
 Pro Tyr Asp His Lys Trp Glu Phe Pro Arg Asn Arg Leu Ser Phe Gly  
 580 585 590  
 Lys Thr Leu Gly Ala Gly Ala Phe Gly Lys Val Val Glu Ala Thr Ala  
 595 600 605  
 Tyr Gly Leu Ile Lys Ser Asp Ala Ala Met Thr Val Ala Val Lys Met  
 610 615 620  
 Leu Lys Pro Ser Ala His Leu Thr Glu Arg Glu Ala Leu Met Ser Glu  
 625 630 635 640  
 Leu Lys Val Leu Ser Tyr Leu Gly Asn His Met Asn Ile Val Asn Leu  
 645 650 655  
 Leu Gly Ala Cys Thr Ile Gly Gly Pro Thr Leu Val Ile Thr Glu Tyr  
 660 665 670  
 Cys Cys Tyr Gly Asp Leu Leu Asn Phe Leu Arg Arg Lys Arg Asp Ser  
 675 680 685  
 Phe Ile Cys Ser Lys Gln Glu Asp His Ala Glu Ala Ala Leu Tyr Lys  
 690 695 700  
 Asn Leu Leu His Ser Lys Glu Ser Ser Cys Ser Asp Ser Thr Asn Glu  
 705 710 715 720  
 Tyr Met Asp Met Lys Pro Gly Val Ser Tyr Val Val Pro Thr Lys Ala  
 725 730 735  
 Asp Lys Arg Arg Ser Val Arg Ile Gly Ser Tyr Ile Glu Arg Asp Val  
 740 745 750  
 Thr Pro Ala Ile Met Glu Asp Asp Glu Leu Ala Leu Asp Leu Glu Asp  
 755 760 765  
 Leu Leu Ser Phe Ser Tyr Gln Val Ala Lys Gly Met Ala Phe Leu Ala  
 770 775 780  
 Ser Lys Asn Cys Ile His Arg Asp Leu Ala Ala Arg Asn Ile Leu Leu  
 785 790 795 800  
 Thr His Gly Arg Ile Thr Lys Ile Cys Asp Phe Gly Leu Ala Arg Asp  
 805 810 815  
 Ile Lys Asn Asp Ser Asn Tyr Val Val Lys Gly Asn Ala Arg Leu Pro  
 820 825 830  
 Val Lys Trp Met Ala Pro Glu Ser Ile Phe Asn Cys Val Tyr Thr Phe  
 835 840 845

WO 03/002107

PCT/IB02/03298

4/5

Glu Ser Asp Val Trp Ser Tyr Gly Ile Phe Leu Trp Glu Leu Phe Ser  
 850 855 860  
 Leu Gly Ser Ser Pro Tyr Pro Gly Met Pro Val Asp Ser Lys Phe Tyr  
 865 870 875 880  
 Lys Met Ile Lys Glu Gly Phe Arg Met Leu Ser Pro Glu His Ala Pro  
 885 890 895  
 Ala Glu Met Tyr Asp Ile Met Lys Thr Cys Trp Asp Ala Asp Pro Leu  
 900 905 910  
 Lys Arg Pro Thr Phe Lys Gln Ile Val Gln Leu Ile Glu Lys Gln Ile  
 915 920 925  
 Ser Glu Ser Thr Asn His Ile Tyr Ser Asn Leu Ala Asn Cys Ser Pro  
 930 935 940  
 Asn Arg Gln Lys Pro Val Val Asp His Ser Val Arg Ile Asn Ser Val  
 945 950 955 960  
 Gly Ser Thr Ala Ser Ser Ser Gln Pro Leu Leu Val His Asp Asp Val  
 965 970 975

<210> 2  
 <211> 30  
 <212> DNA  
 <213> Homo sapiens

<220>  
 <223> Primer

<400> 2  
 aagaagagat ggtacctcga ggggtgaccc

30

<210> 3  
 <211> 33  
 <212> DNA  
 <213> Homo sapiens

<220>  
 <223> Primer

<400> 3  
 ctgcttcgcg gccgcgttaa ctcttctcaa cca

33

<210> 4  
 <211> 20  
 <212> DNA  
 <213> Homo sapiens

<220>  
 <223> Primer

<400> 4

WO 03/002107

PCT/IB02/03298

5/5

agctcgttta gtgaaccgtc

20

&lt;210&gt; 5

&lt;211&gt; 20

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;223&gt; Primer

&lt;400&gt; 5

gtcagacaaa atgatgcaac

20